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Influence of acidulant identity on the effects of pH and acid resistance on the radiation resistance of *Escherichia coli* O157:H7

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Abstract

The effects of pH (4.0–5.5), acid identity (acetic, citric, lactic, malic, and hydrochloric), and the induction of pH-dependent stationary phase acid resistance on the radiation resistance of *E. coli* O157:H7 Ent-C9490 was studied using cells grown in Tryptic Soy Broth with and without dextrose (induced and non-induced to acid resistance) and then resuspended in brain–heart infusion broth containing 5 g/l of an organic acid and acidified with concentrated hydrochloric acid. After treatment with gamma radiation, the number of survivors was determined by plating on brain–heart infusion agar (injured and non-injured cells) and MacConkey agar (non-injured cells), and the data used to calculate radiation *D*-values. The induction of pH-dependent stationary phase acid resistance consistently provided the enterohemorrhagic *E. coli* strain cross-protection from subsequent irradiation, increasing radiation *D*-values by 1.2–3.3-fold, depending on the organic acid present. The radiation resistance of *E. coli* varied with acid identity, but was largely unaffected by pH within the range examined. The results indicate that induction of cross-protection resulting from induction of acid resistance is a factor that should be considered to accurately determine the radiation dose needed to inactivate enterohemorrhagic *E. coli* in foods.

Keywords: Organic acids; Cross-protection; Acid tolerance; Irradiation

1. Introduction

One of the characteristics common among enterohemorrhagic *E. coli* is their relative resistance to acidic environments. This ability to temporarily endure acidic environments is enhanced by the presence of several growth phase related and inducible acid resistance systems, including a pH-dependent system that greatly augments the already considerable acid resistance of stationary phase cells (Buchanan and Edelson, 1996, 1999a; Ryu and Beuchat, 1998; Uljas and Ingham, 1998; Deng et al., 1999; Rowbury and Goodson, 1999; Ryu et al., 1999). This *rpoS*-independent system has been associated with the metabolism of glucose, glutamate, and aspartate in both *E. coli* and *Salmonella* Enteriditis (Buchanan and Edelson, 1996; Rowbury and Goodson, 1999; Wilde et al., 2000). Induction of acid resistance also provides cross-protection against other stresses, including increasing the micro-organism's thermal tolerance (Ingham and Uljas, 1998; Ryu and Beuchat, 1998; Buchanan and Edelson, 1999b; Duffy et al., 2000; Mazzotta, 2001). We have also demonstrated that induction of pH-dependent stationary phase acid resistance provides the micro-organism with cross-protection against gamma radiation (Buchanan et al., 1999). When the effects of pH and acid resistance on the radiation resistance of seven *E. coli* O157:H7 strains were evaluated in microbiological medium adjusted to pH values between 4.0 and 5.5 using hydrochloric acid, differences in pH had a relatively small effect on the micro-organism's radiation resistance. However, regardless of the pH of medium, prior induction of pH-dependent stationary phase acid resistance increased radiation *D*-values by approximately 50%. Similar cross-protection was observed when pH-dependent stationary phase acid resistance was induced in *E. coli* O157:H7 prior to the micro-organism being irradiated in apple juice (Buchanan et al., 1998). These studies

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suggested that irradiation might potentiate subsequent pH-associated inactivation of the pathogen during subsequent refrigerated storage (Buchanan et al., 1999).

Organic acids are generally considered more effective against foodborne pathogens than hydrochloric acid. This increased antimicrobial activity is associated with the anion portion of the molecule and varies among the various organic acids. This general characteristic for bacteria in food holds true for enterohemorrhagic *E. coli* (Buchanan et al., 1999). In the pH range between 4.0 and 5.5, we have found that among five acidulants (lactic, acetic, citric, malic, and hydrochloric acids) tested, lactic acid consistently had the greatest activity against enterohemorrhagic *E. coli* and HCl had the least (Buchanan and Edelson, 1999a). However, the degree of activity and the relative effectiveness of the different acidulants varied substantially among the eight enterohemorrhagic strains examined. Other investigators have also observed differences in the antimicrobial activities of various organic acids and have identified pH and temperature as two of the factors that influence the effectiveness of the acidulants against enterohemorrhagic *E. coli* (Uljas and Ingham, 1998; Deng et al., 1999; Ryu et al., 1999).

While the impact of acidulant identity on *E. coli* survival in acidic environments has been examined extensively, less information is available on the effect that acidulant identity has on associated cross-protection resulting from the induction of acid resistance. This is particularly true for the cross-protection afforded enterohemorrhagic *E. coli* against gamma radiation. The objective of the current study was to expand our earlier work in this area to examine the effect of acidulant identity on (1) the influence of pH on the radiation resistance of *E. coli* O157:H7, (2) the irradiation potentiation of pH-associated inactivation during subsequent refrigerated storage, (3) the ability of pH-dependent stationary phase acid resistance to increase radiation resistance, and (4) the extent of radiation and acid induced injury immediately after irradiation and after subsequent refrigerated storage. Four weak organic acids commonly used in food production, lactic, acetic, citric, and malic acids, were compared against hydrochloric acid, a strong acid with minimal anion effects. The experimentation was conducted in a manner that focused on the effect of acid identity on the cross-protection against gamma irradiation and not on the relative ability of the different acids to induce acid resistance.

2. Materials and methods

All experiments were conducted using the minor modifications of the techniques described by Buchanan et al. (1999). The procedures employed are described briefly below.

2.1. Micro-organism

E. coli O157:H7 Ent-C9490 was selected for use in the current study based on it being among the most acid resistant and radiation resistant isolates in our previous studies (Buchanan and Edelson, 1996, 1999a; Buchanan et al., 1998, 1999). The isolate was originally isolated from an outbreak of hemorrhagic colitis associated with ground beef. The micro-organism was cultured bi-monthly in tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) for 24 h at 37°C and then stored at 2°C.

2.2. Inoculum

The micro-organism was cultured for 18 h at 37°C in TSB w/o dextrose (TSB-G) or TSB + 1% dextrose (TSB + G) to provide stationary phase cells that, respectively, had *rpo*S-associated acid resistance only and *rpo*S-associated + pH-dependent stationary phase acid resistance (Buchanan and Edelson, 1996). It is important to note that TSB w/o dextrose is a separate product from TSB, which contains 0.25% dextrose. That level of dextrose in TSB may be sufficient to induce at least an intermediate level of pH-dependent stationary phase acid resistance.

2.3. Menstrum

Sterile double strength BHI was supplemented with citric, lactic, acetic, or malic acid; adjusted to pH 4.0, 4.5, 5.0, or 5.5 with concentrated HCl; and brought up to volume with sterile distilled water. The final concentration of organic acid was 5 g/l (0.5%). The BHI was transferred in 10 ml portions to two sets of 7 sterile test tubes for each acid-pH-acid resistance induction combination. The tubes were equilibrated at 2°C and maintained at that temperature throughout all-subsequent treatment, storage, and sampling. The tubes were prepared just prior to the initiation of an experimental trial.

2.4. Irradiation

Tubes were inoculated with 0.1 ml of TSB-G or TSB + G culture, and duplicate sets of tubes were treated in a Cesium-137 irradiator at specified doses up to 1.0 kGy. One set of 7 tubes representing six dose levels plus the 0-kGy control was assayed for viable counts immediately and the second was stored for 7 days at 2°C and then assayed. All experiments were performed on at least three separate occasions. Dosimetry was performed using alanine pellets as described in Buchanan et al. (1998).

2.5. Viable counts

After appropriate dilution, samples were plated on duplicate brain–heart infusion agar (BHIA) and MacConkey agar (MA) plates using a spiral plater. The plates were incubated at 37°C for 18–24 h and then enumerated. The BHIA counts were considered the total count (injured and non-injured cells), whereas the MA count provided the non-injured cell count. The number of injured cells was calculated based on the differential between the BHIA and MA counts.

2.6. D-values

Irradiation *D*-values were calculated by taking the negative reciprocal of the slope of the inactivation curve which was determined by linear regression.

3. Results

While occasional survivor curves showed evidence of small shoulders or tails, the irradiation inactivation of *E. coli* O157:H7 followed first-order inactivation kinetics. Linear regressions of the log number of surviving cells versus radiation dose typically had R^2 values >0.95. Thus, a simple linear regression model was used to describe the radiation inactivation of the micro-organism, thereby allowing the use of radiation *D*-values as a direct measure of radiation resistance. Examples of typical survivor curves are depicted in Fig. 1.

The radiation resistance of *E. coli* O157:H7 Ent-C9490 varied to different degrees based on the three parameters being evaluated, i.e., pH, acidulant identity, and induction of acid resistance (Table 1). Radiation *D*-values ranged from a low of 0.051 kGy (non-induced, malic acid, pH 4.5) to a high of 0.214 kGy (induced, HCl, pH 5.0). Within an organic acid—acid resistance induction combination, pH had relatively little, if any, effect on radiation *D*-values. Substantial differences in radiation *D*-values were observed among the different acidulants. When pH-dependent stationary phase acid resistance was not induced, radiation resistance in relation to acidulant identity was lactic acid > citric acid > acetic acid ≅ hydrochloric acid > malic acid. The radiation resistance ranking observed with cells after prior induction of pH-dependent stationary phase acid resistance was hydrochloric acid > malic acid > lactic acid ≅ citric acid > acetic acid. In general, greater differences in radiation resistances among the different acidulants were observed with the cultures induced for

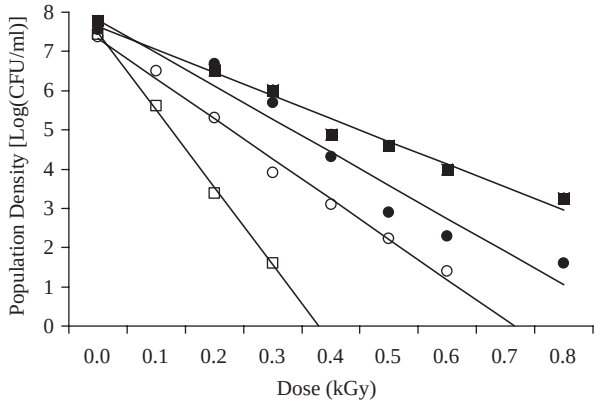

Fig. 1. Examples of typical radiation inactivation curves observed with *E. coli* O157:H7 Ent-C9490 during the course of the experimentation. (■) Malic acid, pH 4.5, induced; (□) malic acid, pH 4.5, non-induced; (●) acetic acid, pH 4.0, induced; (○) acetic acid, pH 4.0, cells non-induced.

Table 1
The effects of pH, acid identity, and induction of pH-dependent stationary phase acid resistance on the radiation *D*-values of *Escherichia coli* O157:H7 Ent-C9490 irradiated at 2°C in BHI containing various organic acids

Acidulant[a]	pH-dependent stationary phase acid resistance	pH			
		4.0	4.5	5.0	5.5
Citric	Induced[b]	0.120[c] (0.008)	0.116 (0.004)	0.137 (0.016)	0.139 (0.007)
	Non-induced	0.088 (0.013)	0.092 (0.012)	0.094 (0.013)	0.099 (0.012)
	RRR[d]	1.36	1.26	1.46	1.40
Lactic	Induced	0.127 (0.023)	0.118 (0.020)	0.140 (0.008)	0.137 (0.003)
	Non-induced	0.104 (0.010)	0.094 (0.018)	0.102 (0.013)	0.100 (0.012)
	RRR	1.22	1.26	1.37	1.37
Acetic	Induced	0.118 (0.029)	0.104 (0.018)	0.103 (0.016)	0.111 (0.010)
	Non-induced	0.096 (0.011)	0.088 (0.014)	0.086 (0.012)	0.084 (0.013)
	RRR	1.23	1.18	1.20	1.32
Malic	Induced	0.173 (0.010)	0.169 (0.006)	0.177 (0.008)	0.174 (0.004)
	Non-induced	0.065 (0.002)	0.051 (0.001)	0.056 (0.002)	0.065 (0.003)
	RRR	2.66	3.31	3.16	2.68
Hydrochloric	Induced	0.207 (0.005)	0.210 (0.004)	0.214 (0.001)	0.207 (0.009)
	Non-induced	0.078 (0.002)	0.086 (0.006)	0.091 (0.004)	0.089 (0.004)
	RRR	2.65	2.44	2.35	2.33

[a] All organic acids were added at concentrations of 5 g/l, with the pH adjusted to the target level using concentrated hydrochloric acid.

[b] Cells were grown in TSB + 1% dextrose and TSB + 0% dextrose for 18 h at 37°C to provide cells that were and were not induced for pH-dependent stationary phase acid resistance, respectively.

[c] Mean (standard deviation). *D*-values based on BHIA counts of samples assayed immediately after irradiation.

[d] Radiation resistance ratio = $D_{(TSB+G)}/D_{(TSB-G)}$.

pH-dependent stationary phase acid resistance. The induction of pH-dependent stationary phase acid resistance by prior growth in an acidogenic (acid producing), glucose-containing medium consistently resulted in greater radiation resistance than that observed with corresponding cells grown in a non-acidogenic (non-acid producing) medium without glucose. The extent of this increase in radiation resistance appeared to fall into two groups based on acidulant identity. Acetic, lactic, and citric acid cultures had radiation resistance ratios ($D_{induced}/D_{non\text{-}induced}$) in the range of 1.18–1.46, while the malic and hydrochloric acid cultures had ratios >2.33 (Table 1). This difference in values appeared to be due, in part, to hydrochloric acid and malic acid both having lower radiation resistance in the non-induced cells and greater radiation resistance in the induced cells compared to the other three acidulants.

The effect of pH, acid identity, and induction of pH-dependent stationary phase acid resistance on the survival of *E. coli* O157:H7 Ent-C9490 during refrigerated storage was evaluated by comparing the levels of the micro-organism in the 0-kGy samples assayed on Day-0 against those treated identically but stored for 7 days at 2°C (Table 2). The cultures induced to acid resistance were largely unaffected by the storage period at the four pH values. The greatest decrease in viable counts was only Log(cfu/ml) = 0.2. The effect of refrigerated storage had a greater impact on the survival of the non-induced cultures. Differences among the acidulants were observed; the cultures were least affected by acetic acid and most affected by malic acid. In general, the micro-organism survived refrigeration best at pH 4.5. While the non-induced cells were clearly more sensitive to the period of refrigerated storage period than the corresponding cultures of induced cells, the non-induced cells were still capable of surviving well during refrigerated storage. With the exception of the pH 5.5—malic acid samples that had a decline of 1.6 log cycles, the decrease in viable counts was less than Log(cfu/ml) = 1.0.

Earlier studies with multiple strains of enterohemorrhagic *E. coli* O157:H7 (Buchanan et al. 1999) that used HCl as the acidulant to assess the effects of pH-dependent stationary phase acid resistance on the radiation resistance suggested that irradiation may potentiate the inactivation of the micro-organism as a result of subsequent refrigerated storage under moderately acidic conditions. As a means of further assessing these earlier observations, two complete sets of cultures were irradiated; one that was analysed immediately after irradiation and used to derive the *D*-values presented in Table 1 and a second set that was held for 7 days at 2°C prior to analysis. Radiation *D*-values for this second set of samples were used as a means of evaluating the combined effects of the irradiation and the stress of the subsequent refrigerated storage (Table 3). The *D*-values calculated with the 7-day data (Table 3) for the cultures induced for acid resistance were generally less than or equivalent to the *D*-values calculated using the 0-day data (Table 1). Since the levels of cells in the 0-kGy acid resistance induced samples did not differ greatly, a decrease in *D*-values after refrigerated storage of irradiated cells would indicate that the exposure of the cells to radiation increased their sensitivity to the subsequent stress of refrigerated storage. However, any differences observed were small, indicating that this was a relatively minor effect. Conversely, the 7-day based *D*-values for the non-induced cultures (Table 3) were generally equivalent to or greater than the corresponding 0-day based *D*-values (Table 1). If the levels of cells in the non-irradiated samples had remained constant, this could be interpreted as indicating that irradiation increased the subsequent survival of *E. coli* during refrigeration storage under moderately acidic conditions. However, the levels of non-induced, non-irradiated cells declined up to 1.6 log cycles (Table 2), which could increase the apparent *D*-values calculated using the 7-day data if the stress of the storage conditions had a greater impact on the non-irradiated cells. This possibility was evaluated further by estimating the differential in viable counts for irradiated cells assayed

Table 2
Decrease in *Escherichia coli* O157:H7 Ent-C9490 levels in 0-kGy samples resulting from the subsequent storage of samples at 2°C for 7days

Acidulant[a]	pH-dependent stationary phase acid resistance	pH			
		4.0	4.5	5.0	5.5
Citric	Induced[b]	⩽0.0[c]	⩽0.0	⩽0.0	⩽0.0
	Non-induced	0.2	0.1	0.4	0.4
Lactic	Induced	⩽0.0	⩽0.0	⩽0.0	⩽0.0
	Non-induced	0.3	0.1	0.5	0.8
Acetic	Induced	⩽0.0	⩽0.0	⩽0.0	⩽0.0
	Non-induced	0.2	0.1	0.1	0.1
Malic	Induced	0.1	0.2	0.2	0.2
	Non-induced	0.9	0.4	0.6	1.6
Hydrochloric	Induced	0.1	0.1	⩽0.0	0.1
	Non-induced	⩽0.0	0.2	0.8	0.8

[a] All organic acids were added at concentrations of 5 g/l, with the pH adjusted to the target level using concentrated hydrochloric acid.

[b] Cells were grown in TSB + 1% dextrose and TSB + 0% dextrose for 18 h at 37°C to provide cells that were and were not induced for pH-dependent stationary phase acid resistance, respectively.

[c] Difference in viable counts [Log(cfu/ml)]: for 0-kGy samples assayed on day-0 minus 0-kGy samples stored at 2°C for 7 days. The values represent the differences of the means of at least three independent trials.

Table 3
Radiation *D*-values for *Escherichia coli* O157:H7 Ent-C9490 based on samples had been stored for 7 days at 2°C after treatment

Acidulant[a]	pH-dependent stationary phase acid resistance	pH			
		4.0	4.5	5.0	5.5
Citric	Induced[b]	0.120[c] (0.026)	0.114 (0.011)	0.121 (0.013)	0.128 (0.028)
	Non-induced	0.099 (0.007)	0.094 (0.013)	0.100 (0.010)	0.105 (0.012)
Lactic	Induced	0.108 (0.014)	0.107 (0.013)	0.139 (0.018)	0.138 (0.035)
	Non-induced	0.095 (0.002)	0.102 (0.011)	0.106 (0.008)	0.103 (0.007)
Acetic	Induced	0.089 (0.011)	0.089 (0.016)	0.092 (0.021)	0.109 (0.017)
	Non-induced	0.095 (0.002)	0.095 (0.009)	0.090 (0.006)	0.091 (0.005)
Malic	Induced	0.176 (0.006)	0.185 (0.005)	0.183 (0.010)	0.169 (0.002)
	Non-induced	0.087 (0.005)	0.073 (0.003)	0.069 (0.001)	0.059 (0.002)
Hydrochloric	Induced	0.196 (0.004)	0.192 (0.008)	0.180 (0.005)	0.173 (0.008)
	Non-induced	0.097 (0.005)	0.095 (0.007)	0.092 (0.004)	0.096 (0.004)

[a] All organic acids were added at concentrations of 5 g/l, with the pH adjusted to the target level using concentrated hydrochloric acid.
[b] Cells were grown in TSB + 1% dextrose and TSB + 0% dextrose for 18 h at 37°C to provide cells that were and were not induced for pH-dependent stationary phase acid resistance, respectively.
[c] Mean (standard deviation). *D*-values based on BHIA counts of samples stored for 7 days at 2°C after treatment and then assayed.

immediately and after 7 days of refrigerated storage using the calculations described by Buchanan et al. (1999). This technique uses linear regression to estimate the dose needed to reach the lower limit of detection for the 7-day samples. This value is then used to determine the corresponding viable count in the samples assayed immediately after irradiation (Table 4). These values were then compared with the values for the non-irradiated samples (Table 2). A value for irradiated samples that was substantially higher than the corresponding non-irradiated sample value would indicate that the exposure to irradiation enhanced the inactivation of the micro-organism during subsequent refrigerated storage. There were a few specific combinations of pH and acid identity (e.g., acetic acid: pH 4.0 and 4.5) that may have had slightly greater decreases in viable counts as a result of the 7-day storage at 2°C. However, overall the data did not indicate that prior low dose irradiation would be a general means of potentiating the inactivation of enterohemorrhagic *E. coli* during subsequent exposure to moderately acidic conditions.

All viable counts were performed using both BHIA and MA as a means of estimating the extent of injury resulting from the various stresses to which the cells were subjected (Buchanan and Edelson, 1999a; Buchanan et al., 1999). The BHIA counts provide a measure of both injured and non-injured cells, whereas MA contains bile salts that hampers the growth of injured cells. Potentially, using viable count data that does not adequately detect injured cells could lead to substantial underestimation of a micro-organism's radiation resistance. However, the ratio of the *D*-values derived from

Table 4
Calculated differential in *Escherichia coli* O157:H7 Ent-C9490 viable counts for irradiated samples assayed immediately after irradiation and after storage at 2°C for 7days

Acidulant[a]	pH-dependent stationary phase acid resistance	pH			
		4.0	4.5	5.0	5.5
Citric	Induced[b]	⩽0.0[c]	0.1	0.7	0.5
	Non-induced	⩽0.0	⩽0.0	⩽0.0	⩽0.0
Lactic	Induced	0.9	0.5	⩽0.0	⩽0.0
	Non-induced	0.8	⩽0.0	0.2	0.6
Acetic	Induced	1.6	0.9	0.7	⩽0.0
	Non-induced	0.2	⩽0.0	⩽0.0	⩽0.0
Malic	Induced	0.1	⩽0.0	⩽0.0	0.5
	Non-induced	⩽0.0	⩽0.0	⩽0.0	2.1
Hydrochloric	Induced	0.4	0.6	1.0	1.1
	Non-induced	⩽0.0	⩽0.0	0.7	0.3

[a] All organic acids were added at concentrations of 5 g/l, with the pH adjusted to the target level using concentrated hydrochloric acid.
[b] Cells were grown in TSB + 1% dextrose and TSB + 0% dextrose for 18 h at 37°C to provide cells that were and were not induced for pH-dependent stationary phase acid resistance, respectively.
[c] Calculated difference in levels of *E. coli* Ent-C9490 for the survivor curves of samples assayed immediately after treatment and those held for 7 days at 2°C. The values were calculated using the technique of Buchanan et al. (1999), using linear regression to determine the dose when the survivor curve for the 7-day cultures reached the lower limit of detection (Log(cfu/ml) = 1.0). This dose was then used to determine the corresponding level of *E. coli* for the cultures assayed immediately after treatment. The difference between the two was then calculated and the value ($= \text{Log(cfu/ml)}_{0\text{-day}} - 1$) was reported.

the BHIA and MA counts from the 0-day samples indicated that there was little difference between the two media (data not shown). Similar results were observed when the *D*-values derived from the BHIA and MA counts for the 7-day samples were compared (data not shown). Examination of the individual survivor curves (data not shown) indicated that the counts on the two media were similar, which is indicative of this strain being resistant to injury.

4. Discussion

The current study builds on our earlier work examining the effects of pH, acid identity, and the induction of acid resistance on the radiation resistance of enterohemorrhagic *E. coli*. The lack of any clearcut effect of pH over the range of 4.0–5.5 on the radiation resistance of enterohemorrhagic *E. coli* is consistent with earlier studies (Buchanan et al., 1999). The possibility that further reductions in pH to values <4.0 might increase the sensitivity of enterohemorrhagic *E. coli* to ionizing radiation awaits further research. However, the results to date suggest that acidification of a food to the pH levels examined in the current study would not be an effective means for decreasing the radiation dose needed to eliminate this pathogen from foods. In fact, acidification of a food might produce the opposite effect if it induced enterohemorrhagic *E. coli* to acid resistance. The current study clearly confirmed earlier work (Buchanan et al., 1998, 1999) that the induction of pH-dependent stationary phase acid resistance cross-protects enterohemorrhagic *E. coli* such that it can better withstand a subsequent exposure to ionizing radiation. This increased resistance was observed with all five of the acidulants (Table 1). However, the extent of the increase in radiation resistance varied among the acidulants, ranging from an 1.2-fold increase in *D*-value with acetic acid at pH 4.5 to a 3.3-fold increase in *D*-value with malic acid at pH 4.5.

The radiation resistance of *E. coli* O157:H7 varied in relation to the acidulant that was present in the BHI being irradiated (Table 1). For the cells not induced to pH-dependent stationary phase acid resistance, the most sensitive cells (*D*-values ranging from 0.051 to 0.065 kGy) were those suspended in BHI containing malic acid. It is worth noting that malic acid also had the greatest effect on non-induced cells that were held at 2°C for 7 days (Table 2). The *D*-values for non-induced cells in BHI containing the other acidulants ranged from 0.078 to 0.104 kGy. The conditions that lead to the greatest observed radiation resistance with cells induced to pH-dependent stationary phase acid resistance were the cultures suspended in BHI acidified only with hydrochloric acid (*D*-values ranging from 0.207 to 0.214 kGy). Interestingly, the next most resistant condition was induced cells suspended in malic acid containing BHI (*D*-values ranging from 0.169 to 0.173 kGy). The other acidulants had *D*-values in the range of 0.103–0.140 kGy. Determination of how the acidulants influence the radiation resistance of the cells to varying degrees will require further research. However, it is important to note that the experimental design of the current study was specifically developed so that acidulant identity was not a factor in the induction of acid resistance. How radiation resistance would have been affected if different organic acids had been used to induce acid resistance awaits further study. Likewise, the current study only examined the acids at a single concentration of 5 g/l, and further work will be needed to determine if the effect of the different acidulants on radiation resistance is affected by concentration. It is also worth noting that the current results are for a single strain of *E. coli* O157:H7. Previous studies examining the acid resistance of enterohemorrhagic *E. coli* have demonstrated substantial variation among strains in relation to their relative sensitivity to different acidulants under acidic conditions (Uljas and Ingham, 1998; Buchanan and Edelson, 1999a; Deng et al., 1999; Ryu et al., 1999). It is possible that other strains would have different profiles in relation to the effect of acidulant identity on radiation resistance. The strain used in the current study, *E. coli* O157:H7 Ent-C9490, was selected because it was among the most acid and radiation resistant strains observed in earlier studies (Buchanan and Edelson, 1996, 1999a; Buchanan et al., 1999).

Little inactivation of *E. coli* Ent-CC9490 was observed during the 7-day period of storage at 2°C in both the non-radiated (0-kGy samples) and irradiated tubes. The ability of this micro-organism to survive these moderately acidic conditions is not surprising. These results are consistent with prior investigations that have observed that enterohemorrhagic *E. coli* can survive for extended periods at pH values similar to or less than those used in the current study, particularly when the pathogen is held at refrigeration temperatures (Ingham and Uljas, 1998; Ryu and Beuchat, 1998; Uljas and Ingham, 1998). The increased survivability of the non-irradiated samples as a result of the induction of pH-dependent stationary phase acid resistance is consistent with the results of our early study (Buchanan et al., 1999). That earlier study suggested that there may be some enhancement of inactivation during refrigerated storage after prior exposure to low-dose irradiation, and similar results were observed in the current study with the cultures acidified with HCl acid. However, this effect was not observed with all acidulants. The effect was relatively minor in the current study, which is not surprising considering that *E. coli* Ent-C9490 was one of the strains least affected in our earlier work because of its extreme acid resistance (Buchanan et al, 1999). Thus, additional studies may be needed to fully characterize

the potential ability of irradiation to accelerate the acid inactivation among different strains of the micro-organism.

The differential plating on BHIA and MA has been very effective in previous studies in identifying the conditions that lead to injury of *E. coli* (Buchanan and Edelson, 1996, 1999a; Buchanan et al., 1999). However, in the current study the extent of injury observed was limited as reflected in the minimal differences in *D*-values observed when *D*-values were derived using viable count data based on plating samples onto BHIA or MA. This likely reflects the fact that *E. coli* Ent-C9490 has been one of the enterohemorrhagic *E. coli* isolates that has consistently displayed minimal injury when exposed to stresses such as irradiation or highly acidic conditions (Buchanan and Edelson, 1999a; Buchanan et al., 1999). For example, it was the least susceptible to injury among 9 strains exposed to pH 3.0 for 7 h at 37°C in the presence of hydrochloric, lactic, acetic, citric and malic acids (Buchanan and Edelson, 1999a).

Low dose ionizing radiation is increasingly being accepted as an effective means of reducing the risk of pathogenic bacteria in foods. However, at the doses currently approved, knowledge of factors that might increase the radiation resistance of the target bacterium or decrease the effectiveness of the treatment is important to assuring the effectiveness of this technology. It is clear from the current study that avoiding the induction of pH-dependent stationary phase acid resistance is among the factors that should be considered to optimize the irradiation treatment of foods.

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